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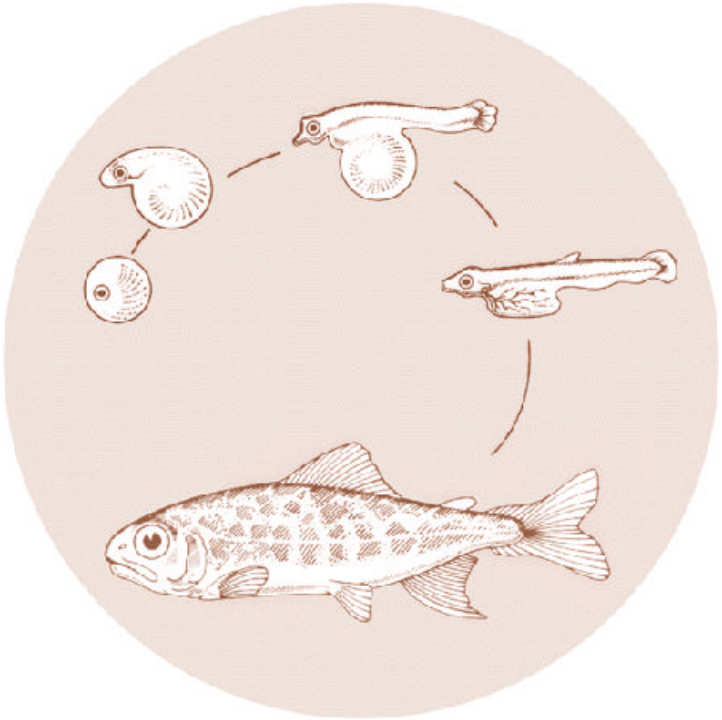
EPIDEMIOLOGY AND CONTROL OF INFECTIOUS DISEASES OF SALMONIDS IN THE COLUMBIA RIVER BASIN

Annual Report FY 1986



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Epidemiology and Control of Infectious Diseases of Salmonids in the Columbia River Basin

Annual Report FY 1986

by

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ABSTRACT

The Department of Microbiology at Oregon State University with funding from the Bonneville Power Administration conducted a study relating to the epidemiology and control of three fish diseases of salmonids in the Columbia River Basin. These three diseases were ceratomyxosis caused by the myxosporidan parasite Ceratomyxa Shasta, bacterial kidney disease, the causative agent Renibacterium salmoninarum, and infectious hematopoietic necrosis, caused by a rhabdovirus. Each of these diseases is highly destructive and difficult or impossible to treat with antimicrobial agents.

The geographic range of the infectious stage of C. Shasta has been extended to include the Snake River to the Oxbow and Hells Canyon Dams. These are the farthest upriver sites tested. Infections of ceratomyxosis were also initiated in the east fork of the Lewis River and in the Washougal River in Washington. Laboratory studies with this parasite failed to indicate that tubeficids are required in its life cycle. Bacterial kidney disease has been demonstrated in all life stages of salmonids : in the eggs, fry, smolts, juveniles and adults in the ocean, and in fish returning to fresh water.

Monoclonal antibodies produced against R. salmoninarum demonstrated antigenic differences among isolates of the bactertum. Monoclonal antibodies also showed antigens of R. salmoninarum which are similar to those of a wide variety of gram positive and gram negative bacteria.

A demonstration project at Round Butte Hatchery showed UV treatment to be an effective method for reducing the microbial population of the water supply and could reduce risks of IHNV. Tangential flow filtration was used successfully to concentrate IHNV from environmental water. At Round Butte Hatchery the carrier rate of IHNV in adults was very low and there was no subsequent mortality resulting from IHN in juveniles.

ACKNOWLEDGEMENTS

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Cooperators in this study are: the Portland General Electric Company, through their representative Mr. Don Ratliff, which owns Round Butte Hatchery and purchased the ultraviolet sterilization equipment; the personnel of the Oregon Department of Fish and Wildlife (ODFW) who operate Round Butte Hatchery, the ODFW also supplied the different stocks of fish and coordinated livebox placement; Dr. Warren Groberg, virologist of the ODFW who assisted in experimental design and sampling at Round Butte Hatchery, and personnel of the Washington Department of Game, Washington Department of Fisheries, Idaho Department of Fish and Game, Army Corps of Engineers, National Council for Air and Stream Improvement, Bureau of Reclamation, Pacific Power and Light, and the Grant County Public Utility who helped coordinate the placing of liveboxes at selected Columbia River and Snake River Dams. Individuals who allowed access to their private property were Bob Wood, Sam Gallus, Bub Weik, Orin Achus, Bob Corrothers and Vance Farris.

INTRODUCTION

Successful propagation and enhancement of fisheries resources requires control of fish pathogens. This study, which has been funded by the Bonneville Power Administration since 1983, focuses on three of these disease agents: Ceratomyxa Shasta, Renibacterium salmoninarum and infectious hematopoietic necrosis virus.

Ceratomyxa Shasta

Prior to this project Ceratomyxa Shasta was known to exist in the mainstream Columbia River to its confluence with the Deschutes River. Live box studies, designed to more precisely define the geographic range of the infective stage of & Shasta within the Columbia Basin, have detected this parasite upriver in the mainstream Columbia River to the confluence with the Snake River. Distribution studies completed this year have detected the parasite as far up the Snake River as Oxbow Hatchery, approximately 597 river miles from the Columbia River bar. These results mean that migrating upriver salmonids are exposed to C. Shasta for a much longer period than previously believed.

The nature of the infectious stage of & Shasta, like most other myxosporidan parasites, is unknown. Recent research with Nyxosoma cerebralis, another myxosporidan infecting salmonid fish, has suggested the oligochaete, tuhifex, acts as an intermediate host in its life cycle. However, our attempts to transmit ceratomyxosis to susceptible fish by exposing them to tubifex incubated with infected viscera and spores have been unsuccessful. Other continuing experiments involve efforts to visualize the infective stage of C. Shasta by using fluorescently labelled antihodies directed against spores of this parasite and to transmit this disease in the laboratory in

tanks containing mud from areas where C. Shasta is endemic and sterilized mud seeded with tubificid worms.

Materials and Methods

Experimental Animals

Ceratomyxa Shasta - susceptible rainbow trout (Salmo gairdneri) were obtained from Oak Springs Hatchery. Those fish which were used to study the geographic distribution of the parasite were held at either the Oregon State University Fish Disease Laboratory (OSU-FDL) or at Wallowa Hatchery near Enterprise, OR. Fish for all other studies were held at the OSU-FDL.

Exposure to Ceratomyxa Shasta

Procedures for exposure to, and detection of, C. Shasta are described by Fryer (1984). All groups of fish used in the geographic distribution study were exposed at selected sites for 14 days. The maximum temperature and the dates of exposure were recorded (Table 1). After exposure all fish were transported to Round Butte Hatchery-Isolation Facility (RBH-IF) and held until termination 120 days later. The water temperature at RBH IF was 10°C.

Fish infected for use in transmission studies of C. Shasta were exposed for five days to the infectious stage of C. Shasta in the Willamette River near Corvallis, OR, then returned to the OSU-FDL and held at 12°C.

Labelling and Evaluation of Antisera

Antisera produced in 1984 and 1985 against spore and prespore stages of C. Shasta were evaluated for their efficacy in detecting the parasite by gel diffusions. Lots of antisera which reacted strongly against the antigen were

labelled with fluorescein isothiocyanate (Banner et al., 1986). Labelled antisera were evaluated for their usefulness in detecting early life stages of C. Shasta.

Collection of Water Samples and Invertebrates

Six, 115 liter samples of Willamette River water and one, 115 liter sample of Cowlitz Hatchery water were differentially filtered using a tangential flow molecular filtration unit (Pellicon Cassette System, Millipore Corp.) with a pore size of 0.5 um. Materials concentrated by this procedure were injected intraperitoneally into susceptible rainbow trout and samples were fixed in formalin for examination by bright light microscopy and fluorescent antibody techniques. Equal volumes of water were collected at the same time, and susceptible rainbow trout were held in them for 6-8 hours to determine if the water was infectious. Both injected and exposed fish were maintained in pathogen-free water and any mortalities were examined for presence of C. Shasta.

Invertebrate samples were collected from sites where water samples were taken. Collections were made using a dip net, an Eckman dredge, and by hand. Organisms were identified to genera and preserved in formalin for examination by fluorescent antibody techniques.

Transmission of Ceratomyxa Shasta Infective Stage

Rainbow trout infected with C. Shasta were placed with unexposed, susceptibile rainbow trout in 15 liter tanks containing several different substrates. These substrates consisted of mud from areas where C. Shasta is endemic (LaCamas Lake and Willamette River), mud sterilized by autoclaving, and sterilized mud seeded with oligochaete worms. All infected fish were fin

clipped and after death from ceratomyxosis allowed to decompose in the tanks. As they died, all unmarked fish were examined for spores or prespore stages of the parasite.

In addition to these small controlled environments, two 100 liter tanks containing mud from the Cowlitz Salmon Hatchery and reservoir below the hatchery and a 500 liter tank containing mud from the Willamette River were prepared. Only uninfected fish were added to these tanks. Fish dying in these tanks will be examined fresh and if they show signs of infection, will be allowed to decompose in the tank.

Preparation of Fluorescent Tagged Antibodies

Polyclonal antisera to C. Shasta has been prepared in New Zealand white rabbits. Lots of antisera which formed precipitin bands against& Shasta in gel diffusions were labeled with fluorescein isothiocyanate (Fryer 1984). All lots of labeled antisera were evaluated by their effectiveness in identifying C. Shasta trophozoites. Evaluation has been done by direct incubation of labeled antisera with trophozoite stages and subsequent visual examination. Western blots (Burnette 1981) of antisera prepared against trophozoite and spore stages have been done on protein profiles of trophozoites and spores. Results of the Western blots and visual evaluation indicate that antisera formed against the spore stage of the parasite may be directed against antigens not present in early trophozoites. Therefore, although the antisera produced are efficient in detecting spores and late trophozoite stages, they have little sensitivity for earlier stages.

Results and Discussion

Geographic Distribution of *Ceratomyxa Shasta*

During the summer of 1986, distribution studies of the infective stage of *C. Shasta* were done primarily in Columbia River tributaries in Washington and in the Snake River and its tributaries in Idaho. The infective stage has now been determined to exist in the Snake above Hells Canyon at Oxbow Hatchery, the highest point on this river system examined (Table 1). Fish exposed in the Washougal and in the east fork of the Lewis Rivers also became infected with *C. Shasta.*

Investigation into the Nature of *Ceratomyxa Shasta* Infective Stage

Samples of Willamette River water containing the infective stage of *C. Shasta* were diffferentially filtered using tangential flow procedures, and all materials larger than 0.45 um were either injected into susceptible rainbow trout or preserved for examination. As a control, an equal volume of water was collected with each sample, and susceptible fish exposed to this water to determine its relative infectivity. Of the seven samples collected, fish in four of the control samples developed ceratomyxosis. Disease did not occur in any group of fish injected with material that had been differentially filtered.

Concentrations of these water samples have been preserved and examined by light and fluorescent microscopy. No spore or prespore stages have been identified, but samples that have been determined to be infective by exposure are still being examined.

Table 1. Incidence of Ceratomyxa shasta in susceptible rainbow trout (Salmo gairdneri) exposed for 14 days in the Columbia River and tributaries during June and July of 1986.

Tributary	Site	River Mile	Maximum Temperature	Date In	Date Out	Number[1] Recovered	Number Dead	Number Infected	Percent Positive	Number of Survivors	Percent Positive
Control	-	-	10.0	7/15	11/5	60	0	0	-	60	0
Salmon	Graves Cr.	37.5	19.0	7/1	7/15	46	0	0	-	46	0
S.F. Clearwater	Stites	6.6	17.0	7/1	7/15	41	0	0	-	41	0
N.F. Clearwater	Dworshak	0.3	11.0	7/1	7/15	52	28[2]	0	0	24	0
Elokoman	Hatchery	5.5	17.0	6/9	6/23	60	0	0	-	60	0
Kalama	Modrow Trap	2.8	18.0	6/9	6/23	60	0	0	-	60	0
E. Fk. Lewis	LaCenter	3.1	22.0	6/10	6/24	59	1	1	100	58	0
Washougal	Camas	1.4	23.0	6/10	6/14	49	7	7	100	42	2.4
Snake	Hells Canyon	246.8	21.3	7/5	7/19	55	53	53	100	2	0
N. Fk. Lewis	Hatchery	15.7	11.0	6/10	6/24	60	0	0	-	58	0
Snake	Oxbow Hatchery	269.8	21.0	7/5	7/19	37	37	35	95	0	0
Columbia	McNary Dam	292	19.0	7/3	7/17	46	23	1	4.4	23	0
Snake	Little Goose	70.3	20.5	7/3	7/17	16	0	0	-	16	0
Clearwater	NCASI Intake	4.5	20.0	7/1	7/15	59	0	0	-	59	0
White Salmon	P.P.L. Plant	2.0	14.0	6/13	6/27	56	0	0	-	56	0
Yakima	Roza Dam	127.9	17.0	6/13	6/27	60	0	0	-	60	0
Wind	Achus Property	5.2	17.0	6/12	6/26	60	0	0	-	60	0
Klikitat	Weik Property	10.4	18.0	6/12	6/26	55	2	0	0	53	0

[1]Number of fish exposed minus holding mortality.

[2]IHN virus isolated from these fish.

Aquatic invertebrates have been collected from the Willamette River, identified, and preserved (Table 2). To date none have been observed to carry stages of C. Shasta.

In artificial environments using mud and aquatic organisms from areas endemic for C. Shasta, infected and control fish have been held in efforts to transmit ceratomyxosis. Transmission has occurred in tanks containing material from the Willamette River. No transmission has occurred in tanks seeded with oligochaete worms from an uninfective source, mud from LaCamas L., or a control tank containing sterilized mud. Artificia environments using mud and invertebrates from Cowlitz Salmon Hatchery and reservoir have also been established and fish are being observed for development of ceratomyxosis.

Renibacterium salmoninarum

Renibacterium salmoninarum, the causative agent of bacterial kidney disease (BKD) is recognized as a major bacterial infection of salmonids. Problems caused by this bacterium extend throughout Columbia River Basin fish hatcheries. Renibacterium salmoninarum was detected, during 1983, in 12% of the chinook salmon smolts seined from the Columhia River just before entering the estuary. These observations served to further delineate the continued economic impact of this pathogen after smolts are released from hatcheries. Limited data in the literature also suggest that BKD continues to cause mortality after salmonid smolts enter salt water. Salmonids caught in the open ocean off the coast of Oregon and Washington have contained BKD lesions and harbored the organism. Since sampling began in 1981, R. salmoninarum has been found by the fluorescent antibody test in 11% of the chinook salmon with lesions in 2.8% of these fish. The presence of lesions is an especially important observation indicating continued progress of the disease and an ongoing open ocean mortality.

Table 2. Invertebrate organisms collected from the Willamette River below Corvallis at river mile 129.6.

Mollusca	
	Pelecypoda
	Nargaritiferidae
	Corbiculidae
	Sphaeridae*
	Gastropoda
	Ammicolidae
	Pleuroceridae
Annelida	
	Oligochaeta
Ephemeroptera	
	Potamanthidae
	Tricorythidae
	Heptageniidae
	Spihlonuridae*
Odonata	
	Gomphidae
Amphipoda	
	Talitridae
Trichoptera	
	Hydropsychidae
	Glossomalidae
Diptera	
	Chironomidae
Coleoptera	
	Elmidae
Hemiptera	
	Corixidae
Turbellaria	
	Planaridae*

*Tentative identification.

Examination by Gram stain and fluorescent antibodies of cryostat-sectioned fertilized eggs collected after one month of incubation in a fish pathogen-free water supply have revealed the presence of bacteria morphologically identical to R. salmoninarum on or in the egg wall. These observations support those of Evelyn et al. (1984) and further suggest vertical transmission of the bacterium.

Knowledge of the serology and antigenic composition of R. salmoninarum is important for the development of reliable serological methods for detection of BKD infections. Although it has been generally accepted there is only one antigenic type of this bacterium (Bullock et al., 1974), there has been limited experimentation done to serologically compare isolates (Getchell et al., 1985). Recent observations indicate there may be more than one antigenic type and that cross-reactions may occur with bacteria from other genera (Austin and Rogers, 1980; Austin et al., 1985). The purpose of developing monoclonal antibodies against Isolates of R. salmoninarum was to produce a reagent to an antigen unique to R. salmoninarum that can be used for immunodiagnosis to eliminate false positive reactions.

Materials and Methods

Experimental Animals and Detection of Renibacterium salmoninarum

Adult salmonids of different year classes, origin, and species were seined off the coasts of Alaska, Washington, and Oregon. These fish were taken by personnel cooperating in a joint research project between Japanese scientists from Hokkaido University in Japan and the School of Oceanography at Oregon State Vniversity. Fish were collected during July 1986 in a cruise of the Oshoro Maru Research vessel. Kidney samples of individual fish were

collected and stored frozen until examined by the fluorescent antibody technique (FAT). Using random numbers tables and methodology, a subsample of 100 individuals of each species captured was selected for examination by FAT. Only 27 chinook were captured and all individuals were examined.

In March 1986, a 100 fish sample of adult spring chinook salmon was taken during the commercial gill net season. The entire kidney of these fish were frozen for later examination. Kidney tissue was homogenized, prepared on microscope slides, and examined by FAT using methods similar to those of Cvitanich (personal communication).

Our previous work (Fryer, 1984, 1985) has indicated that fluorescing bacteria typical of R. salmoninarum can be found in fertilized eggs examined just prior to hatching (after one month of incubation). Attempts to culture the microorganism from these eggs have been unsuccessful. These efforts continued in 1985 by sampling Round Butte Hatchery chinook salmon eggs at frequent intervals during incubation. These eggs were examined by enzyme-linked immunosorbant assays (ELISA) and by culturing egg contents on KDM 2 with charcoal (Daley and Stevenson, 1985).

Production of Monoclonal Antibodies

Monoclonal antibody technology makes it possible to analyze strain specific and cross-reacting antigens among a particular bacterial species. This type of data is necessary for the most effective vaccine production and for the detection of carrier fish.

A modification of the method described by Oi and Herzenberg (1980) was used to produce hybridomas secreting antibody against three strains of R. salmoninarum (Fryer 1984). Strains used were Lea-l-74 (ATCC 33209) (LB) isolated from chinook salmon at Leaburg Hatchery, RB-l-73 (RB) from chinook

salmon at Round Butte Hatchery and K50 from Atlantic salmon (Salmo salar) in Norway. Lymphocytes harvested from mice immunized with R. salmoninarum were fused with SP2 mouse myeloma cells in medium containing 50% polyethylene glycol. After incubation in hypoxanthine, aminopterin, thymidine selective medium, each well of the tissue culture plate containing visible hybridomas was tested by the indirect enzyme-linked immunosorbant assay (ELISA) for production of anti-R. salmoninarum antibody. Hybridoma cultures producing the desired antibody were cloned and assayed twice. Selected hybridomas were grown to be frozen in storage for in vitro assay.

The ELISA used to screen for hybridomas producing anti-R. salmoninarum antibody was modified as described below to increase sensitivity and obtain consistent results. Different concentrations of & salmoninarum cells were attached to 96-well polystyrene microtiter plates (Immulon) previously washed with poly-L-lysine. Test serum or supernatant from cultures containing a hybridoma were added to the wells then incubated with peroxidase-conjugated anti-mouse antibody. A positive culture was detected by a visible color reaction following addition of 0-phenylene diamine substrate. The react ion was quantified by determination of optical density using an automated microplate reader (Biotek EL310). The ELISA was used to detect positive hybridomas and for cross reactivity assays.

Results and Discussion

Prevalence of BKD in Ocean and Columbia River Salmonids

Since 1981 we have been sampling ocean-caught salmonids for the presence of R. salmoninarum. Throughout this study most of the fish captured have been juvenile animals. However, during the summer of 1986, adult salmonids were

seined off the coast of Alaska, Washington, and Oregon. The incidence of BKD in these fish was comparable to that which was determined to be in ocean caught juveniles (Table 3). Chinook salmon had the highest frequency of infection and 19% of those examined carried R. salmoninarum. Pink salmon had a 10% infection rate, 3% of the steelhead that trout examined were infected, chum salmon had 2% incidence and only 1% of the coho and sockeye salmon had detectable levels of R. salmoninarum.

Table 3. Incidence of Renibacterium salmoninarum in adult salmonids captured off the coasts of Alaska, Washington and Oregon, 1986.

Salmonid species	Number examined	Percent positive by FAT[1]
Chinook salmon	27	19
Coho salmon	100	1
Sockeye salmon	100	1
Pink salmon	100	10
Chum salmon	100	2
Steelhead trout	100	3

[1]All fish were examined for R. salmoninarum by the fluorescent antibody test (FAT).

Returning adult spring chinook salmon were sampled as they entered the Columbia River. These fish were taken in the commercial gill net fishery and of 128 fish which were examined 24 or 19% had detectable levels of R. salmoninarum. None of the fish from this sample revealed large numbers of the organism.

Eggs which came from a grossly infected spring chinook salmon at Round Butte Hatchery and fertilized with sperm from an infected male were sampled

for R. salmoninarum at approximately 10 day intervals. Sixty eggs were sampled at each time and the egg contents examined by ELISA and also culture on KDM 2 charcoal. Only one sample was positive by ELISA and five of the eleven samples which were cultured were positive.

Monoclonal Antibody Production

More than 2000 hybridomas have been tested for the stable production of anti-R. salmoninarum antibodies. Numerous clones have been examined and a selected group are presented in Table 4. The antibody produced by these hybridomas has been characterized to class and subclass (Table 5). An

Table 4. Renibacterium salmoninarum isolates and monoclonal antibodies produced from them.

Isolate	Origin	Monoclonals
ATCC 33209 (LEA-l-X)	Fall chinook salmon Oregon	LBll/ll, LB11/9, LB9/8, LB3/5
RB-1-74	Fall chinook salmon Oregon	RClO/l, RC7/2, RF8/9, RG9/6
K50	Atlantic salmon Norway	K34/4, K8/9, K23/15, K11/12, K18/4

unusually high number of these are IgG3 molecules and arise, perhaps, because they are formed in response to carbohydrate containing antigens. Isoelectric focusing has been performed on each of the monoclonal antibodies to determine that they are truly monoclonal.

Table 5. Class and subclass of monoclonal antibodies produced from strains of Renibacterium salmoninarum

Monoconal Antibody		Immunoglobulin Class and Subclass
LB:	LB9/8	IgG2A
	LB11/9	IgGl
	LBll/ll	KgGl
	LE3/5	IgG3
RB:	RClO/l	IgGl
	RC7/2	IgGl
	RF8/9	IgG3
	RG9/6	IgG3
K50:	K23/15	IgG3
	K8/9	IgG3
	K18/4	IgGl

The monoclonal antibodies selected for characterization and testing were reacted against homologous and heterologous antigens of R. salmoninarum (Table 6) with other isolates of this bacterium (Table 7) and

Table 6. Results of ELISA of monoclonal antibodies against heterologous and the homologous strains of Renibacterium salmoninarum.

R. salmoninarum used for fusion	Monoclonal Antibody Designation	Antigen		
		K50	RB	LB
K50	K34/4	+		+
	K8/9	+		+
	K23/15	+		+
	K11/12	+		+
	K18/4	+	+	+
RB	RClO/l		+	+
	RC7/2		+	+
	RF8/9		+	+
	RG9/6		+	+
LB	LB11/11		+	+
	LR11/9		+	+
	LB9/8		+	+
	LE3/5		+	+

Table 7. ELISA and IFAT of Renibacterium salmoninarum monoclonal antibodies against selected isolates of the bacterium.

Origin of Isolate	Monoclonal K18/4 IFAT	K18/4 ELISA	LB IFAT	LB ELISA	RB IFAT	RB ELISA	K50 IFAT	K50 ELISA
Oregon (5 strains)	+	+	4/5+	4/5+	4/5+	4/5+	-	-
Washington	+	+						
Alaska	+	+						
British Columbia	+	+						
Alberta (6 strains)	+	+						
Nova Scotia	+	+						
England	+	+						

with antigens of diverse gram negative and gram positive organisms (Table 8). Antibody-antigen reactions have been tested both by ELISA and an indirect

Table 8. Reaction of selected bacterial isolates with a monoclonal antibody (K18/4) produced against an isolate of Renibacterium salmoninarum.

Isolate	IFAT	ELISA
Aeromonas salmonicida	+	+
Pseudomonas fluorescens	+	+
Yersinia enterocolitica	-	+
Bordetella sp.	-	-
Corynebacterium xerosis	+	-
Corynebacterium pseudodiphtheriticum	-	-
Corynebacterium pyogenes	ND	-
Bacillus subtilis	ND	+
Staphylococcus aureus	+	+
Streptococcus lactis	+	+
Lactobacillus piscicola	ND	+

fluorescent antibody test (IFAT). From the data it can be determined that we have produced a monoclonal antibody which will react with cellular antigens of numerous bacteria, ones that are very different from R. salmoninarum. These results offer an explanation for the cross reactivity and nonspecificity that workers have experienced when using serodiagnostic tests for BKD. We have also shown that different antigenic types of R. salmoninarum exist because some of the monoclonals will not react with all isolates of the bacterium.

Infectious Hematopoietic Necrosis Virus

Infectious hematopoietic necrosis virus (IHNV) has recently become more widespread in the Columbia River Basin and has caused severe losses among chinook salmon and steelhead trout at several Columbia River Basin fish hatcheries. No anti-IHNV drugs are known; therefore, management techniques to avoid the virus, especially during the egg incubation and fry stages, are being tested. Since 1983 at Round Butte Hatchery, we have used UV-treated water for rearing of fish and have also selected eggs and sperm from virus-free adults.

During the spawning of steelhead trout in 1984 at Round Butte Hatchery a unique and potentially very important observation was made. On three separate occasions IHNV was detected in ovarian fluid samples after storage for 6-9 days at 4'C. No virus had been detected in these samples when collected at spawning. Routine sampling for IHNV requires only the processing of tissues and sex fluids taken at spawning; however, this delayed appearance of virus indicates that sampling only at spawning may yield false negatives. These observations raise the possibility that IHNV is more widespread among salmonid populations than previously considered. Further, the production of IHNV by constituent(s), probably cellular, in ovarian fluid represents a novel method for studying the biology of IHNV.

Materials and Methods

Virus Propagation and Detection

Procedures for virus propagation have been described previously (Fryer, 1984). Chinook salmon embryo (CHSE-214) and epithelioma papillosum cyprini (EPC) cell lines were continuously cultured in Eagle's minimum essental medium (MEH) supplemented with fetal calf serum (10%), NaHC03 (0.075%), penicillin (100 iu/ml>, streptomycin (100 pg/ml) and glutamine (1.0%). The EPC MEM growth medium was buffered with Tris hydrochloride instead of NaHC03. Growth temperatures were 16°C for CHSE-214 cells and 22°C for EPC cells.

Plaque assay procedures as described previously (Fryer, 1984) were similar to Burke and Mulcahy (1980). Briefly, assays were performed using confluent EPC monolayers grown in multi-well tissue culture plates. Samples were diluted in Hank's balanced salt solution (HBSS). Replicate 0.1 ml samples (10^{0}–10^{-4}) were inoculated onto monolayers in individual wells and allowed to adsorb for 60 min. Sample inoculum was removed and 1% methyl-cellulcse dissolved in double strength MEM plus 5% fetal calf serum overlay medium (MEM-5) was added. Following 10 days of incubation at 16°C cells were fixed with FORMALIN and stained with 1% crystal violet solution. Plaques were counted and plaque forming units per ml (PFU/ml) were determined in replicate wells containing 10-300 plaques.

Ovarian and seminal fluids were collected and processed as previously described (Fryer, 1984) except supernatant fluids were mixed 1:l with an antibiotic solution (McDaniel 1979) before inoculation onto cells. Ovarian fluid samples were also passed through 0.22 UM acrodisc filters (Gelman) to obtain cell-free preparations. Tissue cells from ovarian fluid samples were

cultured in 75 cm^2 tissue culture flasks in a media consisting of $NaHCO_3$ buffered MEM plus 10% fetal calf serum with antibiotics (McDaniel, 1979).

Monitoring of the Water Supplies at Round Butte Hatchery

During the IHNV studies conducted at Round Butte Hatchery, the water supplies to experimental lots of fish were monitered for the presence of microorganisms. Water was tested before and after UV treatment. There was continuous monitoring of the UV treatment system to insure that the radiation output was always within the manufacturer's specifications remaining continuously above 68% on the UV monitor. Turbidity measurements of hatchery water were recorded daily to determine if siltation could be affecting the UV treatment system. Readings were taken using a Spectronic 20 at 520 um using nanopure water as the control. No siltation was detected in any of the samples.

Water samples from Round Butte Hatchery were collected at selected times from January through May, 1986. During this period, ten samples of the hatchery water supply, prior to UV treatment and immediately after UV treatment, were taken. Bacterial plate counts were performed immediately following sample collection. Spread plates of 1.0, 0.25 and 0.1 ml water samples in triplicate were made using trypticase soy agar (Difco) and cytophaga agar (Anacker and Ordal, 1959). Plates were incubated for 3-5 days at 16°C and colonies were counted.

The tangential flow filtration method of virus concentration was applied to field studies. The Pellicon cassette system which had been evaluated previously under laboratory conditions (Fryer, 1985) was used. The exclusion size of the membranes was 1000,000 molecular weight. Based on data gained from our laboratory experiments with virus stabilizing agents, fetal bovine serum (FBS) (0.1%) was added to each water sample to improve virus recovery.

Fifty liters of water was collected in a sterile carboy, supplemented with 0.1% FBS, and transported on ice. Maximum storage of samples before filtering was two days at 4°C. The 50 1 was concentrated by tangential flow filtration to approximately 100 ml. These samples and the retentate and backflush solutions collected from each 50 liter aliquot were concentrated further by ultracentrifugation. The pellet from this step was resuspended in a small volume of tissue culture fluid and inoculated onto EPC and CHSE-214 cells. If there was no evidence of virus after 14 days, the supernatant was blind-passed onto new cells and observed for cytopathic effect (CPE) for another 14 days.

Design of IHNV Studies at Round Butte Hatchery

The studies with IHNV at Round Butte Hatchery are a demonstration project designed to determine if brood stock selection and/or water treatment reduces the incidence of IHN in fry. In 1986, the initial strategy was to maximize the potential for vertical transmission of IHNV by mating parents with high virus titers and making a comparison between replicate groups of the resulting fry and groups which arose from parents both of which were negative for IHNV. Each group was to be replicated in UV treated and untreated water giving the following lots of fish:

	Number of Replicates	
	Untreated water	UV treated water
IHNV negative parents	4	4
IHNV positive parents	4	4

Fish were spawned on January 29 and February 11, 1986. On each date, 100 females and 125 males were spawned. The gametes of each individual fish were

stored separately while tissue from individual fish were analyzed for the presence and titer of IHNV. Only 6 of the 450 fish (1.3% incidence) were positive for IHNV. The construction of the groups described above was impossible; therefore,the experimental design was altered so that only gametes from negative parents were crossed. Four groups of eggs were incubated and fry reared in UV treated water. Four replicate groups were reared in untreated water (Appendix A). An additional eight groups which arose from production spawning on Marhc 11, 1986 were divided and incubated and reared in the same way (Appendix B). This resulted in eight groups from negative parents which were reared in treated water and eight similar groups reared in untreated water.

Results and Discussion

The demonstration project at Round Butte Hatchery resulted in some positive observations, although the major hypothesis could not be tested. The results demonstrated that IHN is yet to be a predictable disease even though a facility may have a historical record of it. The number of adults carrying the virus and fry or fingerling mortality fluctuate from year to year. This year at Round Butte Hatchery there was a very low carrier rate in adults and there was no mortality in juveniles as a result of IHNV.

It was shown that the tangential flow filtration method of concentrating IHNV from environmental water is an efficacious technique. We isolated IHNV from one sample of nontreated water. We also demonstrated that the treatment of water with UV irradiation at this facility can be an effective method of reducing microorganisms in the water (Table 9).

Table 9. Monitoring of Round Butte Hatchery Water 1986.

Date	Water sample	Bacterial Count TSA	Bacterial Count Cytophaga agar	Virus recovery
Jan. 29	W-treated	<30	<30	+
	non-treated	<30	<30	
Feb. 11	W-treated	<30	<30	
	non-treated	100	129	
Feb. 25	W-treated	<30	<30	
	non-treated	140	150	
Mar. 11	W-treated	44	84	
	non-treated	70	174	
Mar. 25	W-treated	<30	61	
	non-treated	88	222	
Apr. 28	W-treated	<30	61	
	non-treated	57	173	
Apr. 22	W-treated	104	97	
	non-treated	56	148	
May 6	W-treated	<30	<30	
	non-treated	52	81	
May 20	W-treated	136	160*	
	non-treated	68	136	
May 29	W-treated	<30	<30**	
	non-treated	49	140	

Iodophore treated 1 day before samples.

**Chlorine treated 1 hr before samples.

SUMMARY AND CONCLUSIONS

From November 1986 - October 1986, Bonneville Power Administration funded a study concerning epidemiology and control of three infectious diseases affecting salmonids in the Columbia River Basin. These serious fish pathogens are: *Ceratomyxa Shasta*, the causative agent of ceratomyxosis, *Renibacterium salmoninarum*, the causative agent of bacterial kidney diseases and the viral etiology of infectious hematopoietic necrosis.

Ceratomyxa Shasta

The infective stage of *C. Shasta* was determined to be present in the Snake River at Hells Canyon Dam and Oxbow Hatchery. These sites were the farthest upriver location which was tested. Infections with this parasite also occurred at the east fork of the Lewis and the Washougal Rivers in Washington. These observations are a major extension of the known geographic range of *C. Shasta*. That infections of & *Shasta* can occur at Hells Canyon (and possibly above) means that upriver salmonids are exposed to this parasite during a major part of their migration and that many Columbia River salmonid stocks may be in the presence of the infectious stage of & *Shasta* throughout their entire freshwater migration. These data support our previous observations that as many as 12% of the salmonids entering the Columbia River estuary are lethally infected with *C. Shasta*.

Efforts to elucidate the infectious stage of this parasite have included the production of antisera against the spore stage and against ascites fluid which contains pre-spore stages of *C. Shasta*. It was hoped that these reagents would be useful in formulating a fluorescent antibody test for detection of the infectious stage. Antibodies produced detected the spore and certain pre-spore stages of the parasite but not early stages. The reagent

also lacked sufficient sensitivity to be useful in identifying early infections.

Artificially induced challenges using infectious water concentrated by tangential flow filtration or artificial environments containing infected fish and tubificids have shown that the spore stage is not infectious and indicates that tubificids are not required in the development of C. Shasta.

Renibacterium salmoninarum

Several monoclonal antibodies to selected isolates of & salmoninarum have been produced. Using these monoclonals we have determined that there are antigenic differences among strains of the organism which causes bacterial kidney disease. We have also shown that there are antigens of R. salmoninarum which are shared with a wide variety of other bacteria to include gram positive and gram negative organisms. These data give evidence for cross-reactivity and the non-specificity of immunodiagnostic reagents which employ polyclonal or rabbit antisera.

We have confirmed the presence of & salmoninarum in all life stages of salmonids. The bacterium has been detected in eggs and in juveniles in fresh water. Bacterial kidney disease has been shown to be an ongoing problem as fish migrate to salt water and our detection of infections in both subadults and adults in the ocean indicated the disease continues in salt water. Fish still harbor R. salmoninarum when they return to fresh water.

Infections Hematopoietic Necrosis Virus

The numbers of IHNV infected adults was minimal this year and there was no IHNV-caused mortality in fry. This observation and those of previous years demonstrate that IHNV carrier rates in adults fluctuate from year to year.

It was shown that tangential flow filtration is an effective method for concentrating IHNV form the aquatic environment and that at Round Butte Hatchery UV treatment reduces the microbial population of the water supply.

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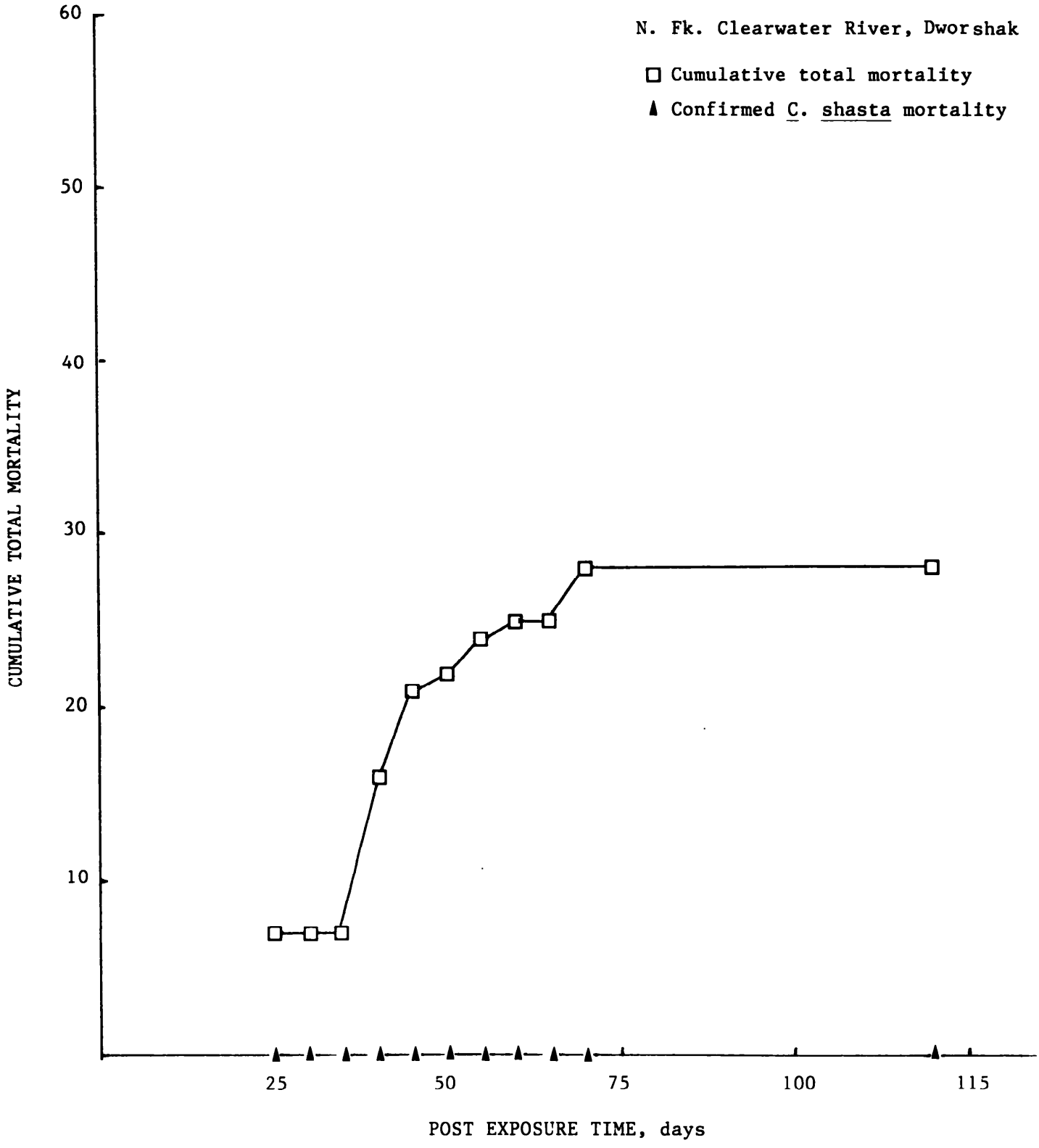
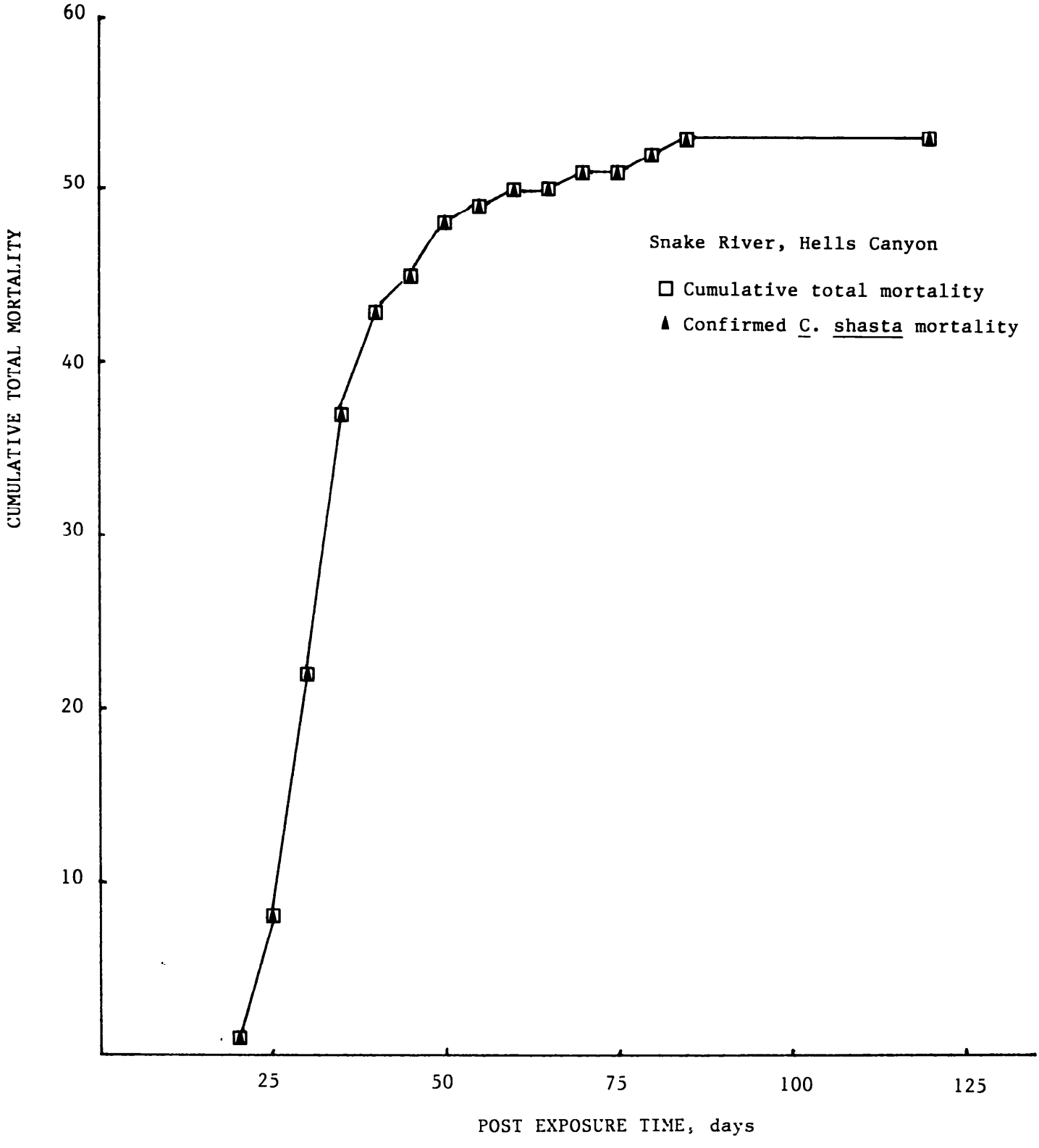
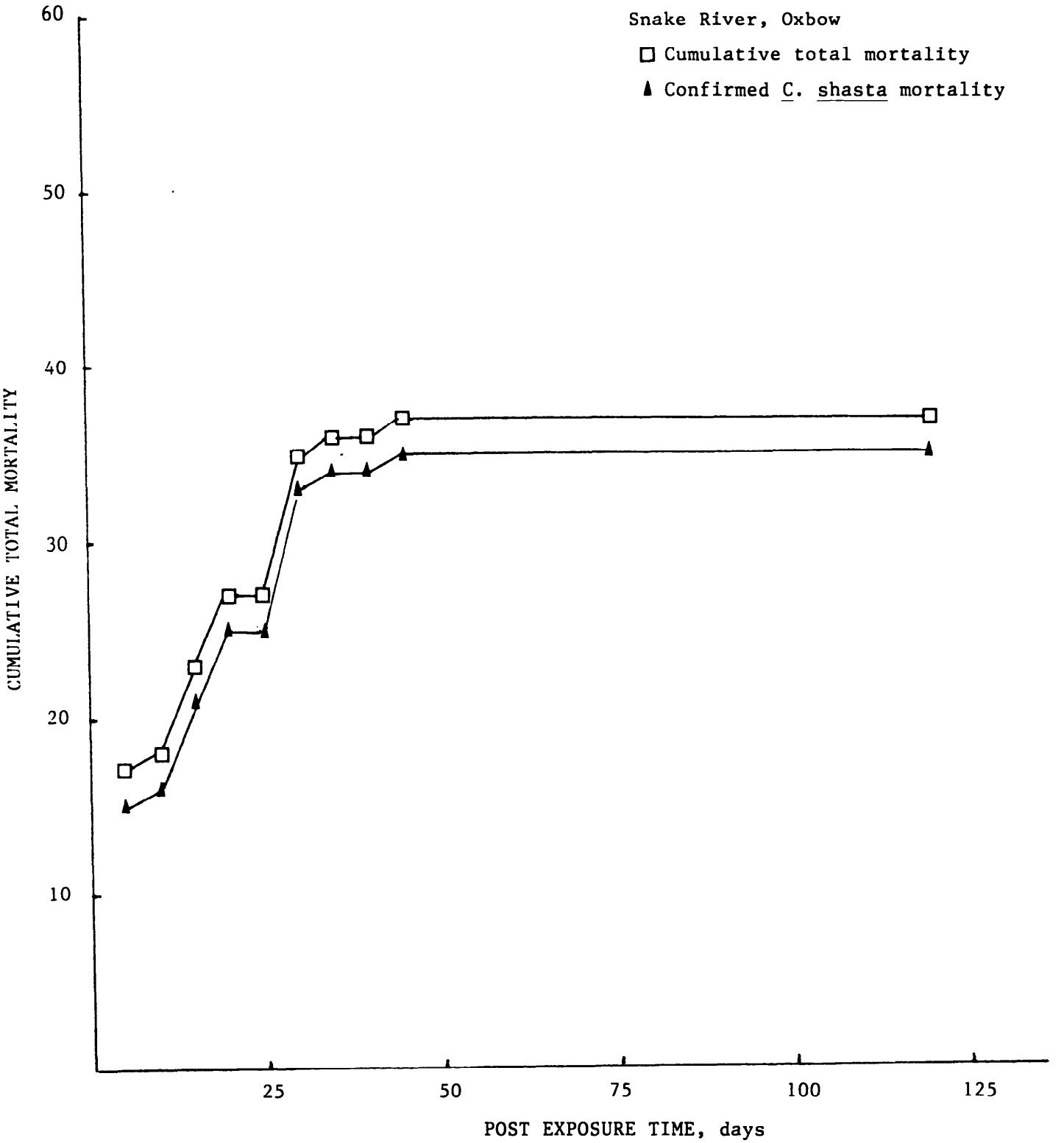
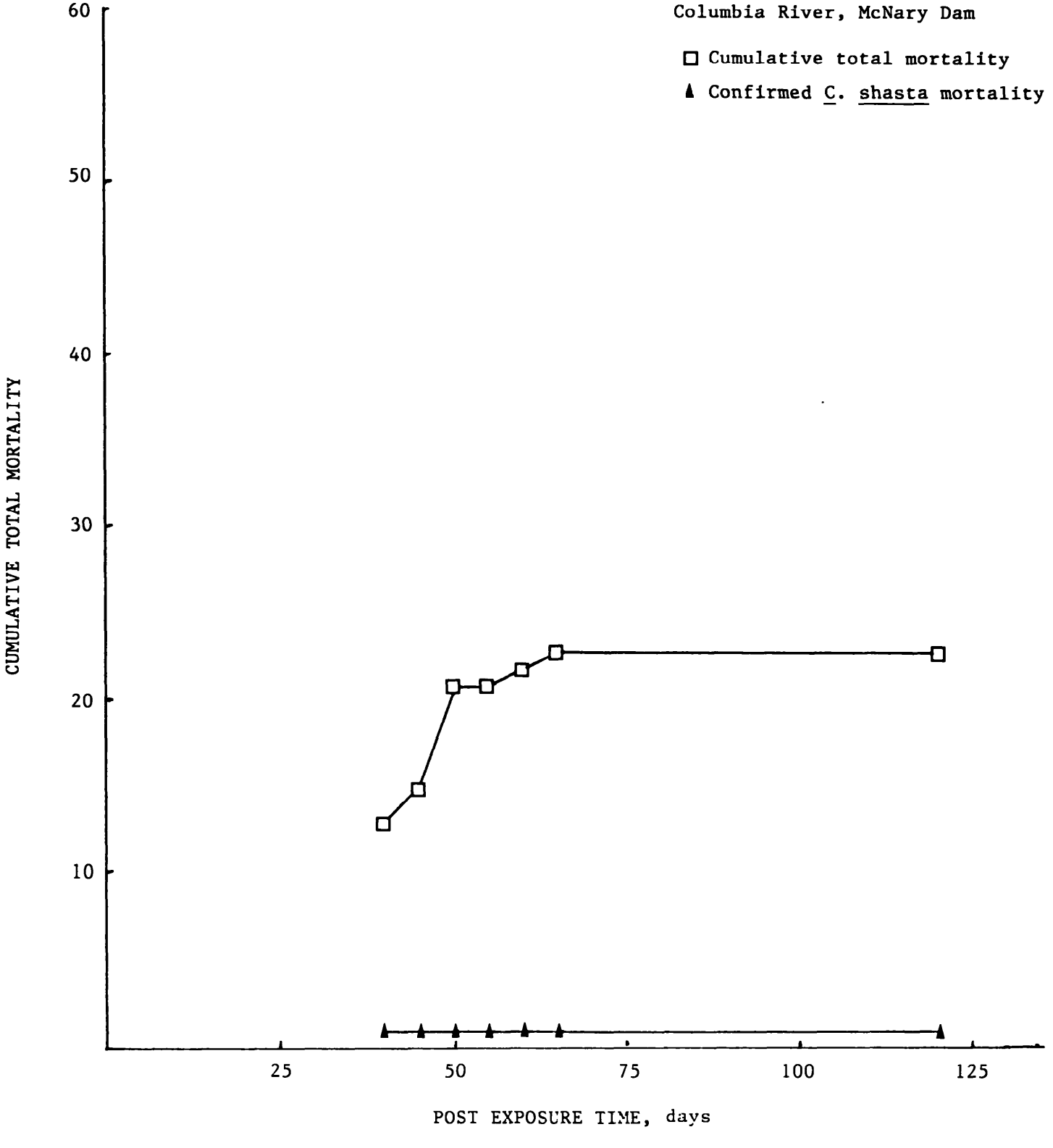
APPENDIX A

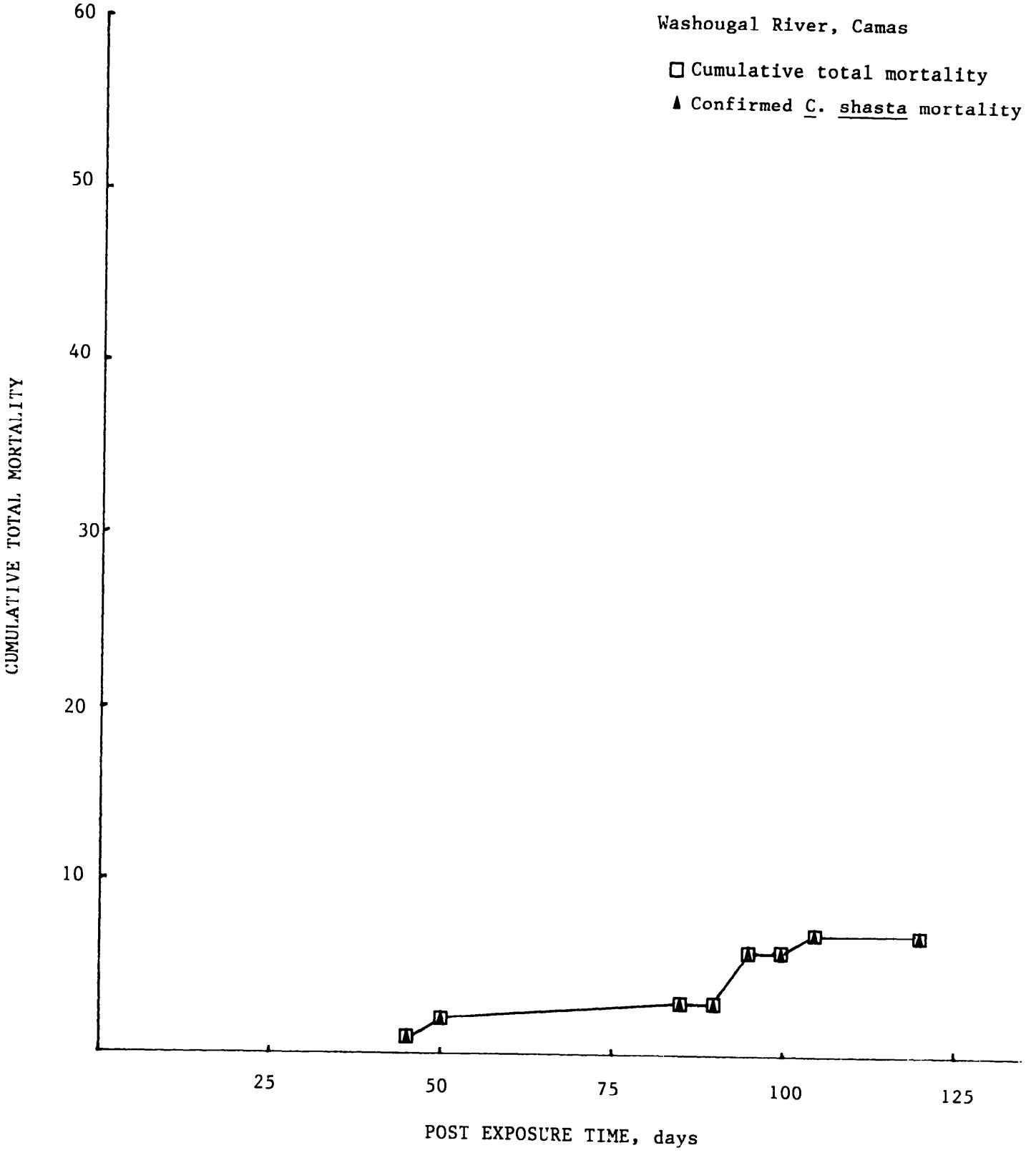
MORTALITY DATA FOR EXPERIMENTS DETERMINING GEOGRAPHIC RANGE OF *CERATOMYXA SHASTA*

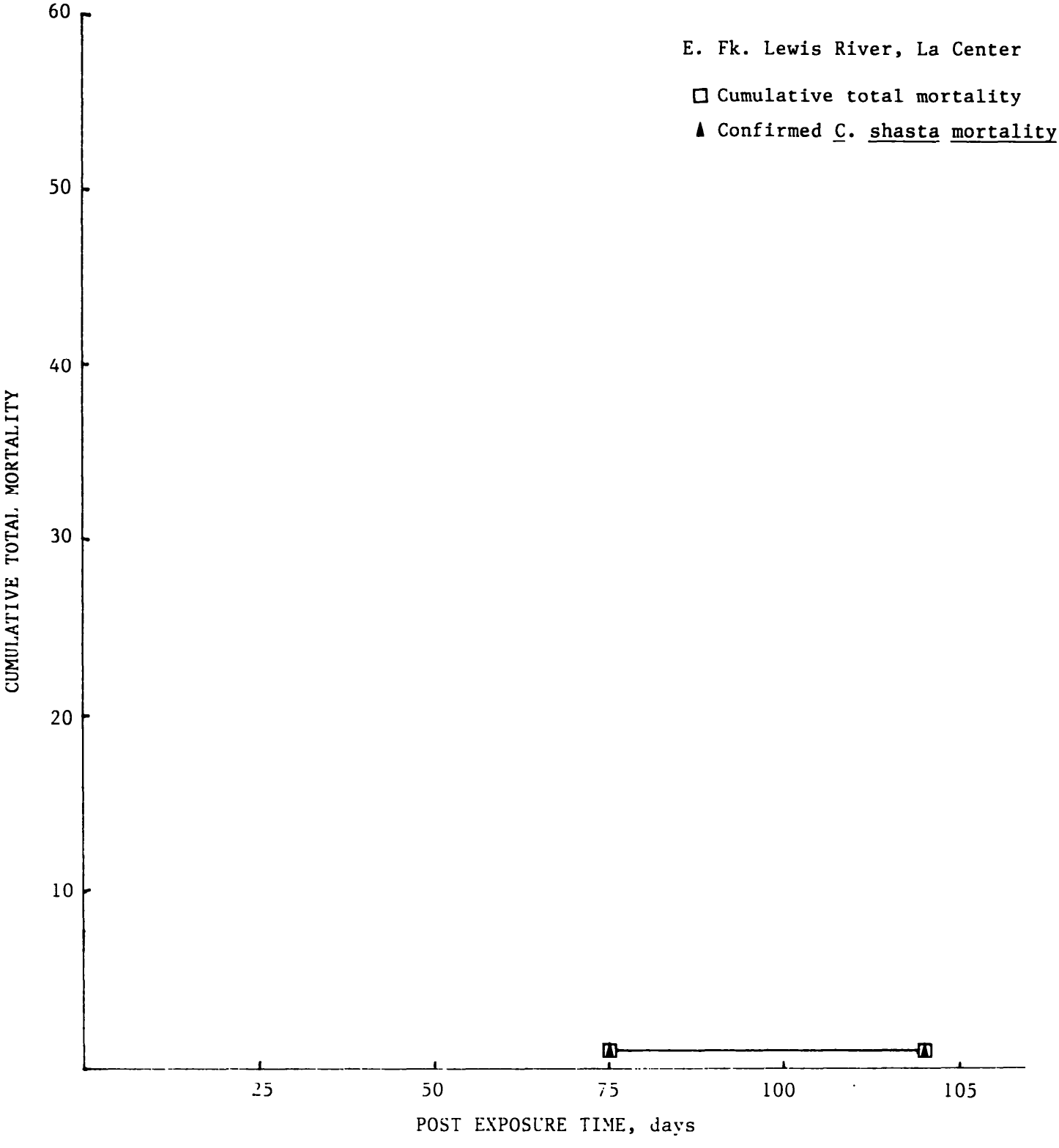

N. Fk. Clearwater River, Dworshak
Cumulative total mortality
Confirmed C. shasta mortality
CUMULATIVE TOTAL MORTALITY
60
50
40
30
20
10
25
50
75
100
115
POST EXPOSURE TIME, days


60
50
40
30
20
10
CUMULATIVE TOTAL MORTALITY
Snake River, Hells Canyon
Cumulative total mortality
Confirmed C. shasta mortality
25
50
75
100
125
POST EXPOSURE TIME, days


Snake River, Oxbow
Cumulative total mortality
Confirmed C. shasta mortality
CUMULATIVE TOTAL MORTALITY
60
50
40
30
20
10
25
50
75
100
125
POST EXPOSURE TIME, days


Columbia River, McNary Dam
Cumulative total mortality
Confirmed C. shasta mortality
CUMULATIVE TOTAL MORTALITY
60
50
40
30
20
10
25
50
75
100
125
POST EXPOSURE TIME, days


Washougal River, Camas
Cumulative total mortality
Confirmed C. shasta mortality
CUMULATIVE TOTAL MORTALITY
60
50
40
30
20
10
25
50
75
100
125
POST EXPOSURE TIME, days

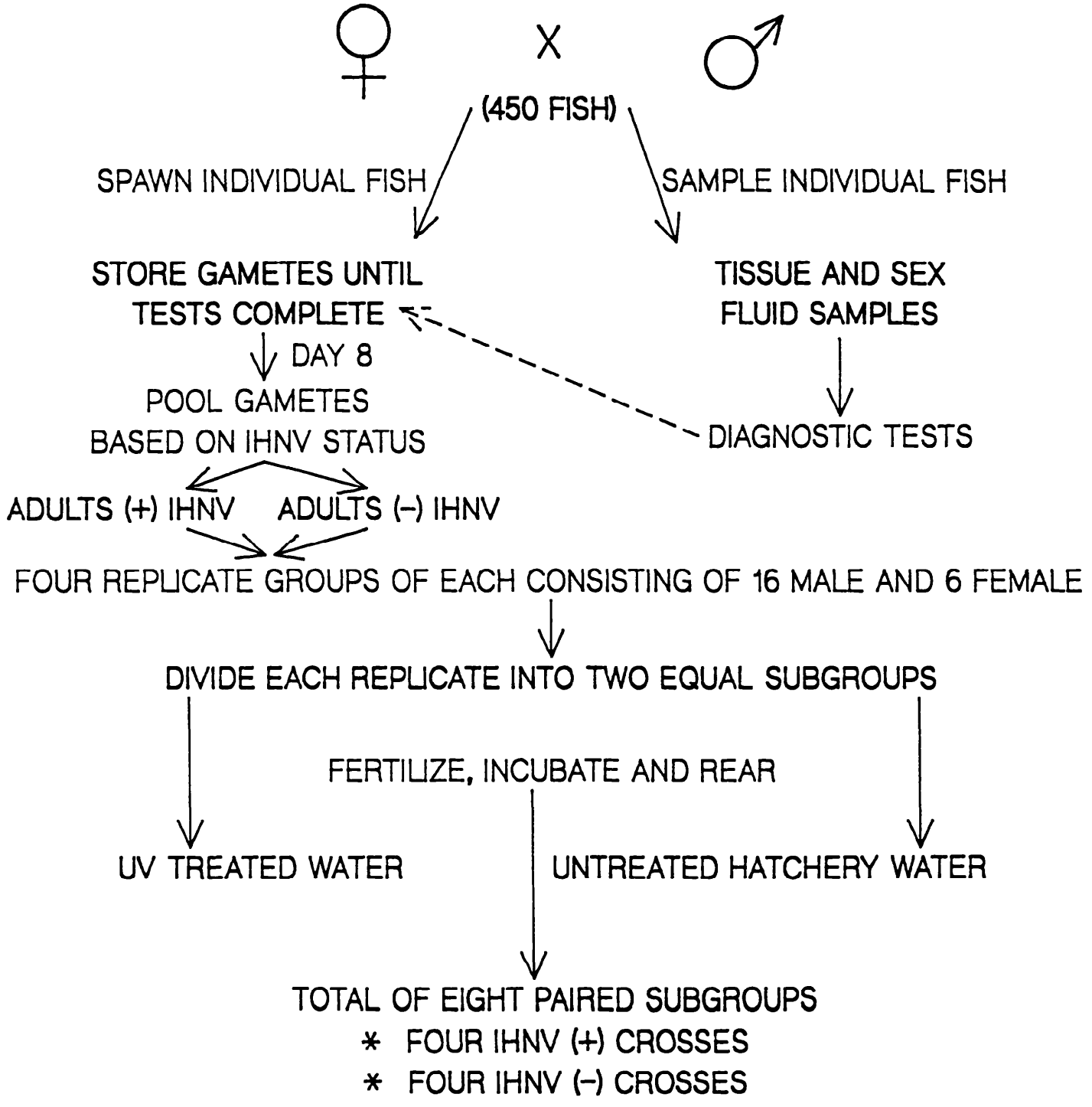

E. Fk. Lewis River, La Center
Cumulative total mortality
Confirmed C. shasta mortality
CUMULATIVE TOTAL MORTALITY
60
50
40
30
20
10
25
50
75
100
105
POST EXPOSURE TIME, days

APPENDIX B

FLOW DIAGRAMS FOR ROUND BUTTE HATCHERY IHNV EXPERIMENTS

1986 IHNV VERTICAL TRANSMISSION STUDY

* VERTICAL TRANSMISSION = INFECTION OF PROGENY VIA VIRUS CONTAMINATED GAMETES

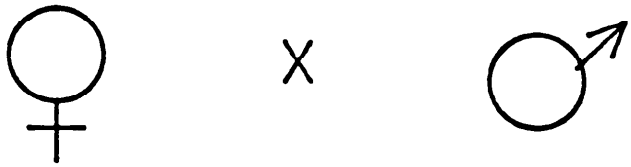


* EXPECTED BENEFIT: DETERMINATION OF THE EXISTENCE OF VERTICAL TRANSMISSION

1986 IHNV HORIZONTAL TRANSMISSION STUDY

* HORIZONTAL TRANSMISSION = INFECTION OF PROGENY VIA VIRUS CONTAMINATED WATER

PRODUCTION CROSSES



(134 FISH)

SPAWN IN 30 FISH POOLS

SAMPLE INDIVIDUAL FISH

TISSUE AND SEX FLUID SAMPLES

DIVIDE INTO TWO SUBGROUPS

FERTILIZE, INCUBATE & REAR

DIAGNOSTIC TESTS

UV TREATED WATER

UNTREATED WATER

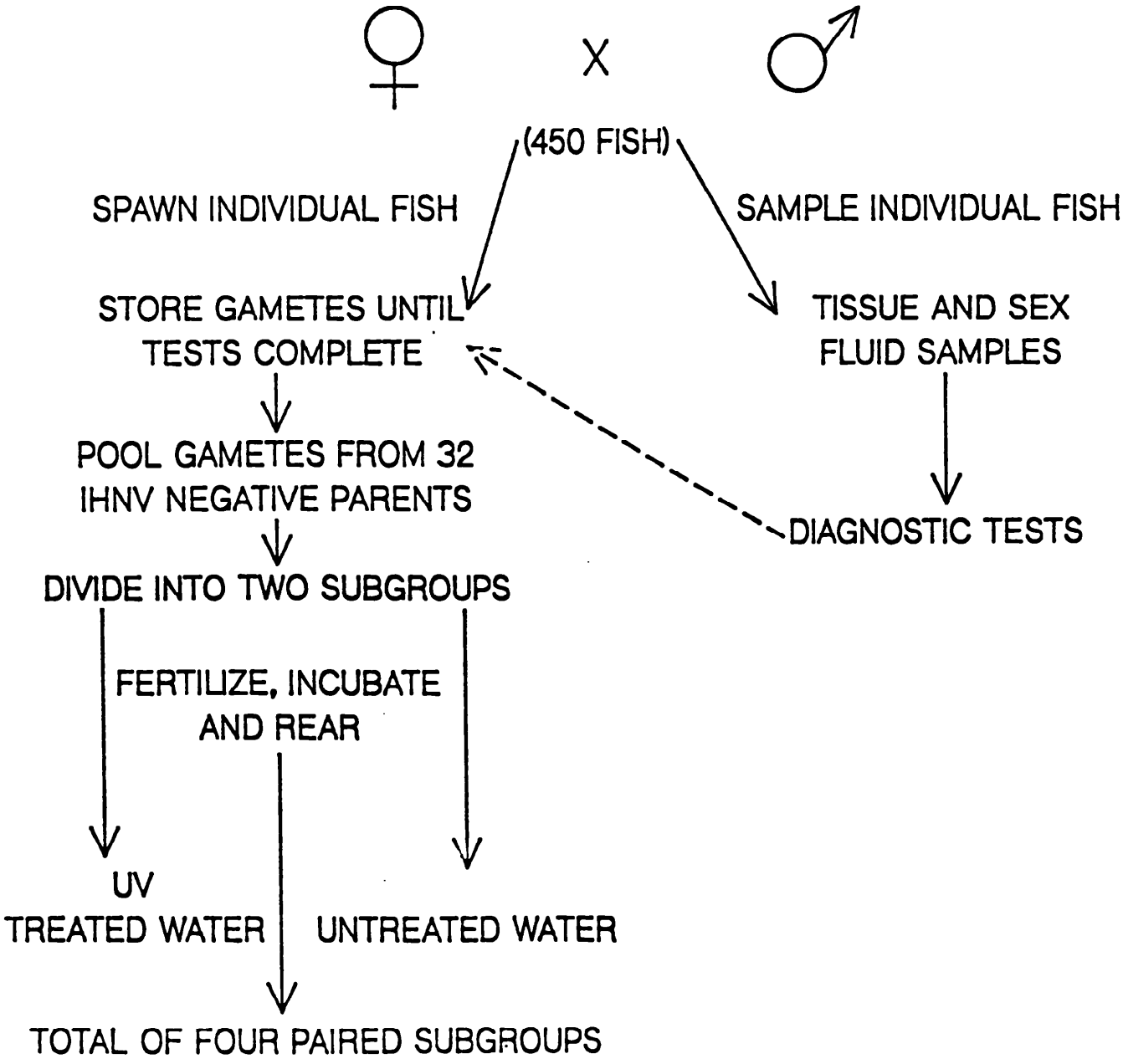
TOTAL OF FOUR PAIRED SUBGROUPS

* EXPECTED BENEFIT: EFFECTIVENESS OF UV STERILIZATION AT PREVENTING FISH LOSS DUE TO THE HORIZONTAL TRANSMISSION OF IHNV

1986 IHNV HORIZONTAL TRANSMISSION STUDY

* HORIZONTAL TRANSMISSION = INFECTION OF PROGENY VIA VIRUS CONTAMINATED WATER

IHNV NEGATIVE MATING PAIR CROSSES



* EXPECTED BENEFIT: EFFECTIVENESS OF UV STERILIZATION AT PREVENTING FISH LOSS DUE TO THE HORIZONTAL TRANSMISSION OF IHNV